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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Maurer, et al.	
Application No.: 10/019,199	
Filed: 12/20/2001	Group Art Unit: 1615
Title: Methods for Preparation of Lipid-Encapsulated Therapeutic Agents	Examiner: G.S. Kishore
Attorney Docket No.: INEX.P-005	Confirmation No: 6234
Customer No.: 021121	

DECLARATION UNDER RULE 132

The undersigned, hereby declare as follows:

I, Michael J. Hope, Ph.D. state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.
2. I am currently a Principal Scientist at Inex Pharmaceuticals Corporation (Burnaby, Canada), a biotechnology company whose primary focus is the development of cancer treatments that are based on its propriety drug delivery platform and that are more effective and have fewer side effects than conventional cancer treatments. I have been a Principal Scientist at Inex Pharmaceuticals Corporation since 1991. Prior to joining Inex Pharmaceuticals Corporation, I was a Vice-President of Research at Canadian Liposome Company (North Vancouver, Canada)
3. In addition, I am currently an Adjunct Professor in the Department of Medicine at the University of British Columbia (Vancouver, Canada). I have been a Professor, either an Adjunct Professor or an Assistant Professor, at the University of Columbia since 1989. I have been a Professor in both the Department of Medicine and the Department of Biochemistry.

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4. In 1973, I graduated from the Queen Elizabeth College, University of London (London, England) with a Bachelor of Science degree in Biochemistry. In 1976, I was awarded my Ph.D. in Membrane Biochemistry from the Royal Free Hospital School of Medicine, University of London (London, England). My graduate studies were carried out under the direction of Professor J.S. Lucy, Head of the Department of Biochemistry, Royal Free Hospital, School of Medicine, and my dissertation was entitled "Effects of Modification of Cholesterol Content on Chemically Induced Fusion in Erythrocytes."
5. Attached hereto as Exhibit A is a true copy of my *curriculum vitae* and a list of publications of which I am an author or co-author.
6. I have read and am familiar with the contents of the above-referenced patent application. In addition, I have read and have reviewed the Office Actions issued by the Examiner, including the Office Action mailed December 30, 2003. This declaration is submitted in support of arguments against the rejection made in the December 30th Office Action.
7. The claims of this application are directed to a method for preparing lipid particles that contain a fully-encapsulated charged therapeutic agent such as an oligonucleotide or polynucleotide. The product formed in this method therefore has the charged therapeutic agent on the inside of the lipid particles, encapsulated in and protected by the lipid. The method involves taking pre-formed lipid vesicles, and combining them with the charged therapeutic agent and a destabilizing agent and incubating for a period of time sufficient to allow encapsulation of the therapeutic agent. Thereafter, the destabilizing agent is removed. The preformed lipid vesicles comprise at least two lipid components: a charged lipid that is opposite in charge to the therapeutic agent, and a modified lipid having steric barrier properties.
8. In the Office Action of December 30, 2003, the examiner has cited three references, US Patent No 6,447,800 of Hope et al., US Patent No. 5,976,567 of Wheeler and WO 98/51278. As a person skilled in the art, I believe that the Examiner is taking selected parts from each of these references in a manner which would not be apparent absent the guidance of the present application.
9. The Hope patent teaches a method for loading materials, including therapeutic agents,

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into liposomes by rendering a preformed liposomal membrane permeable using an organic solvent, preferably ethanol. The key to the Hope patent is that ethanol permeabilizes the membrane without changing the structure. The ethanol establishes an organic-solvent induced permeation gradient (col8, lines 34-65). In order to effectively trap the material in the liposome, after membrane permeation, the permeability barrier must be restored. This is generally accomplished by diluting the solvent (col 9, line 22). Nucleic acid loading methods disclosed in Wheeler dissolve the lipid material in solvent, DNA is then added to initiate formation of the lipid DNA particle.

10. As stated in the Hope patent, the liposomes may be formed from "a variety of vesicle-forming lipids." (Col. 6, line 22 et seq.) The list set forth in the patent does not include cationic lipids.
11. Based on tests that I conducted during the period between 1990-1992, the Hope technique does not work with charged oligonucleotides. Using an ionizable cationic lipid in the present method the inventors have electrostatic interaction for a high D:L ratio but a neutral particle at pH 7.0
12. The therapeutic agents used in the method of the present invention are charged. The Hope patent states that "generally highly negatively charged species such as polynucleotides do not cross the liposomal membranes permeabilized by the solvent technique disclosed herein." (Col. 10, lines 7-9). Without knowledge of the present invention, the idea of adding charged lipids of opposite charge to the therapeutic agent would not be a reasonable option, since one skilled in the art would reasonably expect that adding positive charges in the lipid would cause negatively charged oligo to bind to the lipid, thus changing the liposome structure completely which teaches away from the direction of Hope.
13. US Patent No. 5,976,567 of Wheeler relates to the formation of lipid-nucleic acid complexes. The method makes use of ethanol, but this is not a point of similarity with the Hope patent, because the ethanol is not used to permeabilize a pre-formed lipid membrane. There is no disclosure in Wheeler of introducing nucleic acids, or any other charged material through the membrane of a pre-formed lipid particle. Instead, as reflected in Fig. 40 of Wheeler, formation of the particles results from rearrangement of

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the lipid membrane or from a coating of particles onto the DNA.

14. Wheeler discloses the use of cationic lipids in the lipid nucleic acid particles. The positive charges on these lipids interact to form an ionic interaction with the negatively charged nucleic acids, and the resulting particles are used in transfection. Thus, the lipids of the Wheeler particles interact with cell membranes to facilitate the introduction of materials associated with the lipid particle into the cell. This is a different purpose than the liposomes of Hope, which are not said to be used for transfection.
15. In Wheeler (Col. 2 line 16), there is a reference to introduction of nucleic acids into preformed liposomes to form resultant complexes containing cationic and neutral lipids, but no indication of the source. The cited Behr reference does not support the statement. It is believed that the type of complexes referred to are of the type described by Felgner, and have the following structure



This type of complex is different than the invention of Wheeler because the introduction of a charged therapeutic agent into the lipid bilayer of a preformed particle because there is no control of the size of the resulting particle. The size of a particle containing a therapeutic agent can be a significant factor in toxicity and clearance rate of the particles. Thus, the ability to control it predictably is important and this type of control is lacking in the teaching at column 2, line 16. Whereas in Wheeler, the dissolving of the lipid in solvent and subsequent addition of DNA to facilitate the formulation process of the lipid DNA complex allows for size control. However, Wheeler does not teach encapsulation of the oligo inside the liposome, nor does Wheeler teach the use of solvent for a purpose other than for the dissolution of the lipids.

16. In addition, the type of complex taught in Wheeler at column 2, line 16 is different from the presently claimed invention because there is no organized lipid structure of defined size, and no encapsulation of the oligonucleotide.

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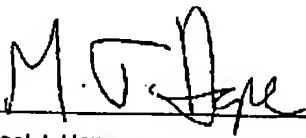
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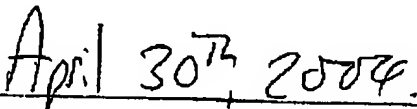
In a manufacturing process using the current method, one can add material to the interior of the liposomes without significantly altering the liposome size.

17. The claims of the present invention also require that the amount of modified lipid that prevents aggregation be an intermediate amount that is sufficient to control aggregation, without completely eliminating it. This intermediate amount is important to the success of the method of the present invention. As noted in the application (Page 13 and Example 7), the formation of the particles of the invention appears to involve an aggregation step, i.e. it is a structural organization process and not a simple permeability effect as in HOPE, and is not a process of simple passage of the charged material, such as a polynucleotide, through the membrane.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Michael J. Hope



Dated